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## DESTRUCTION OF YEAST UNDER CONDITIONS OF SIMULTANEOUS ACTION OF CAVITATION AND ARGON

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### Abstract

The aim of the study was to investigate the cavitation effect and inert gas on the viability of yeast in the water and to determine the gas effectiveness during cavitation treatment of the water system. Experimental data on the simultaneous action of argon bubbled at a rate of 0.2 cm<sup>3</sup>/s through an aqueous medium (volume 75 cm<sup>3</sup>) and ultrasonic cavitation (frequency 22 kHz, power 35 W) on the yeast *Saccharomyces cerevisiae* during the two-hour process are presented. The number of microorganisms per unit volume of test water was determined by the total number of colonies on the nutrient medium on Petri dishes. An active decrease in the number of cells at the beginning of the process (61.84 % after 30 min) with the initial microbiological contamination of water 2.07 × 10<sup>4</sup> CFU/cm<sup>3</sup> with the achievement of the proportion of dead cells > 98 % after water treatment for 1 hour. The obtained results indicate intensive cavitation purification of water from the investigated microorganisms during argon bubbling.

**Key words:** destruction of yeast cells, destruction degree, argon action, cavitation.

**1. Problem statement.** In open water and industrial wastewater, along with impurities of natural origin are different chemical contaminants (pesticides, phenols, petroleum products, salts of heavy metals, etc.), due to the discharge into the reservoir of insufficiently treated or untreated industrial and domestic wastewater [1]. In addition to the existing chemical pollution with organic matter, waste from various industries: petrochemical, pulp and paper, as well as municipal waste, animal waste, biological pollution has a very negative impact on the state of water resources [2]. This type of pollution occurs due to the entry into water bodies together with wastewater of various pathogenic microorganisms, bacteria, fungi, small algae, worms. Several million bacteria are found per unit volume of wastewater and river water. The main sources of biological pollution are municipal wastewater. The volume of bacterial mass at the number of 100 million bacteria in 1 cm<sup>3</sup> is 0.04 % of the wastewater volume [3].

The fight against their mass reproduction in wastewater collection systems, in technological environments, in water supply systems should be aimed at the emergence of new cost-effective water treatment technologies.

A promising method of water purification is the use of cavitation, which is enhanced by the simultaneous bubbling of gases. The effect of inert gases on the viability of microorganisms has not been studied in cavitation conditions. However, the study of the action of inert gases is a great interest for scientific purposes because of their nature, their chemical inertness, because the additional bubbling of gas into the reaction

medium leads to accelerated destructive action of cavitation on microorganisms [4].

### 2. Analysis of the recent researches and publications.

After reviewing the scientific material on the topic of the study, it was found that the cavitation effect on the various microorganism is presented in both domestic and foreign works. The results of such studies are presented in scientific publications [1, 5-11], which investigated the effect of ultrasonic cavitation on algae, fungi, bacteria. The intensity of ultrasonic cavitation on the microorganisms structure in the disinfection wastewater process is described in [8].

The effect of cavitation action on the yeast of *Saccharomyces cerevisiae* is presented in [9], in which ultrasound (US) action of the low frequency (frequency of 28 kHz, power of 140 W/l) lasting 1 hour significantly reduces the number of yeast cells [9].

In [12], the active destruction of *Bacillus cereus* bacteria in an argon atmosphere ( $k_d = (2.3 \pm 0.1) \times 10^{-4} \text{ s}^{-1}$ ) was noted, compared with helium ( $k_d = (8.16 \pm 0.07) \times 10^{-5} \text{ s}^{-1}$ ), regardless of the initial number of bacteria in 1 cm<sup>3</sup> of test water. Since argon has shown high efficiency during microorganisms destruction, it is interesting to study its effect on yeast cells in combination with the cavitation process of water treatment. In addition, yeast differs significantly in structure from bacteria, and we have not found experimental data that would confirm the effect of inert gases under cavitation conditions on yeast cells in the literature.

Yeast contaminates wastewater with products of its vital activity, including products of organic origin. Therefore, the wastewater of the brewing industry is concentrated by organic pollutants, which requires additional treatment before discharge into open water. Therefore, there is a need to find an alternative method of water treatment with a high content of yeast cells, which would purify such water to the level allowed for the discharge of wastewater into water bodies. Therefore, it is proposed to carry out the process of processing yeast cells with the simultaneous action of argon and cavitation, as the joint action of cavitation and gas intensifies the destructive processes on microorganisms in the aquatic medium during its processing [9].

**3. Statement of the problem and its solution.**

The task of the presented research is the following:

- to investigate the change of the yeast cells in the aqueous medium during simultaneous treatment with cavitation and argon;
- to establish the change of destruction degree ( $D_d$ ) from the sonication treatment.

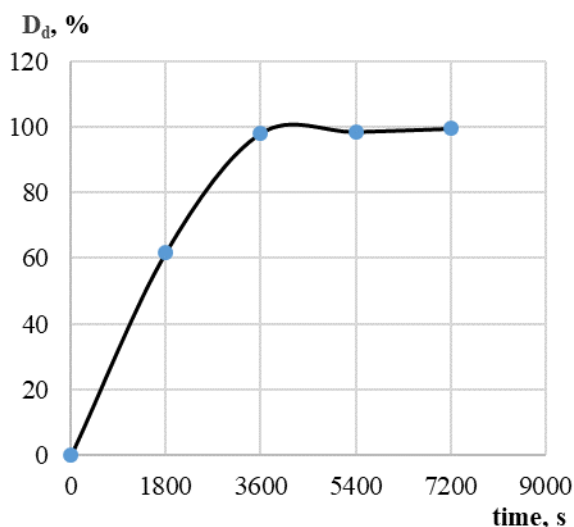


Figure 1 – Dependence of the proportion of destroyed cells on the duration of the simultaneous action of argon and cavitation.

Table 1 – Viability of *Saccharomyces cerevisiae* cells during different treatment regimens

Processing time, s	CFU/cm <sup>3</sup>
0	$2.07 \times 10^4$
1800	$7.9 \times 10^3$
3600	$4 \times 10^2$
5400	$3 \times 10^2$
7200	$1 \times 10^2$

**3.1. Materials and methods.**

*Saccharomyces cerevisiae* yeast was a microscopic objects for research, which was isolated from the wastewater of brewing production. Pure cultures of microorganisms were grown in test tubes under laboratory conditions at 30°C for 96 hours on wort agar, followed by storage at 4°C. Pure culture of microorganisms was added to sterile natural water, thus preparing a suspension of yeast cells in sterile water. The initial number of microorganisms (NM) per 1 cm<sup>3</sup> of test water was  $2.07 \times 10^4$  CFU. The method of diluting microorganisms suspension and the conditions of their cultivation are described in [11].

Experimental conditions were:  $T = 298 \pm 1$  K,  $P = 0.1$  MPa, the total process duration was 2 hours. The source of cavitation was an ultrasonic generator UZDN-2T with a frequency of 22 kHz and a power of 35 W.

The inert gas argon, which has shown high results in the processes of water purification from bacterial cells in our previous studies [12], was selected as the test gas for bubbling of the reaction medium.

The prepared sample of test water was poured into a glass reactor with a capacity of 75 cm<sup>3</sup>, which was constantly cooled by running water to maintain a constant temperature in the reaction medium ( $T = 298 \pm 1$  K). Ultrasound oscillations from the low-frequency generator were transmitted by a magnetostrictive emitter immersed in the volume of the sample water. At the same time including the ultrasonic generator provided argon. The total gas consumption was 1.4 dm<sup>3</sup>, which was fed at a rate of 0.2 cm<sup>3</sup>/s into the studied volume of water throughout the process. Then periodically (every 30 min) water samples were taken for analysis. To summarize the results, this experiment was repeated three times. Statistical processing was performed on the basis of arithmetic mean calculation for a series of experimental data.

NM values were determined by the number of colony forming units (CFU) per unit volume of test water during cell growth on Petri dishes with wort agar. To determine the number of living cells in the samples after treatment, sowing was performed in three parallel Petri dishes from each sample.

**3.2. Results and Discussion.**

Water samples were subjected to simultaneous Ar/US action with an initial yeast content of  $2.07 \times 10^4$  CFU/cm<sup>3</sup>. The sequence of NM changes during water treatment is listed in the Table 1. As we can see, according to tabular data, NM value decreased in 2.6 times after 1800 s of treatment time, and after an hour – in 51.8 times.

The Figure 1 shows a rapid increase in the proportion of dead cells at the beginning of the process, lasting up to one hour. Thus, NM value has decreased significantly after 1800 s, the proportion of destroyed cells is 61.84 %, and an hour later – already 98.07 % (see Figure 1). After an hour of Ar/US processing, the curve reaches the plateau, i.e. the proportion of destroyed cells varies within one.

The final NM did not exceed 100 CFU/cm<sup>3</sup>, and the calculated value of D<sub>d</sub> after two hours of Ar/US treatment was 99.52 % (see Figure 1). These data indicate almost complete water purification and the efficiency of argon bubbling in cavitation conditions, while after Ar/US treatment of water with high content of sporogenic bacteria *Bacillus cereus* (NM<sub>0</sub> = 1.77 × 10<sup>4</sup> CFU/cm<sup>3</sup>) the percentage of destruction is 85.15 % in similar experimental conditions [12].

Thus, when bubbling argon under cavitation conditions managed to achieve almost complete purification of water from yeast. The high efficiency of argon can be justified by the fact that argon is characterized by a higher yield of pyrolysis products [4], due to lower thermal conductivity compared to helium. That is, the saturation of the aqueous medium containing yeast with inert argon leads to the formation of additional cavitation nuclei in the reaction zone, which led to the active microorganisms destruction. Argon also has a lower ionization potential (15.7 eV) than helium (24.5 eV), which greatly facilitates the electronic breakdown in the cavity, promotes more intense decay of water molecules in it, which increases the efficiency of water disinfection. Breaks in flow density with the formation of vapor-gas bubbles occur in places of heterogeneity of the medium, and inhomogeneities are the yeast cells themselves, which in a cavitation explosion (implosion) are found in the

center of cracking [4]. As a result, microorganisms are completely destroyed near the cracking point. According to [1], cavitation action causes significant mechanical destruction of the cell wall, cytoplasmic membrane, the release of intracellular components.

Thus, studies allow us to describe the processes of yeast cells destroyed under the combined action of argon and cavitation. These experimental data are also consistent with the results of our previous experiments [12], according to which the effect of argon on water containing *Bacillus cereus* is described by a higher value of the effective constant of cell destruction: k<sub>d</sub> (Ar) > k<sub>d</sub> (He), due to the nature of the action gas under experimental conditions [12].

#### 4. Conclusion.

The viability of yeast under cavitation conditions and bubbling of argon through the water system has been studied. The share of destroyed cells during two-hour treatment of yeast-contaminated water at different treatment regimes was calculated and compared.

Reduction of microbiological water pollution by two orders of magnitude (from 2.07 × 10<sup>4</sup> to 1 × 10<sup>2</sup> CFU/cm<sup>3</sup>) has been established, which allows discharging treated water into open reservoirs.

Almost complete destruction of *Saccharomyces cerevisiae* in aqueous medium (D<sub>d</sub> = 99.5 %) was noted, which indicates high efficiency of joint Ar/US action in water treatment processes.

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**Коваль І. З.****РУЙНУВАННЯ ДРІЖДЖІВ В УМОВАХ ОДНОЧАСНОЇ ДІЇ КАВІТАЦІЇ ТА АРГОНУ**

Завданням роботи було дослідити вплив кавітації та інертного газу на життєздатність дріжджів у воді та визначити результативність дії газу під час кавітаційної обробки водної системи. Наведено експериментальні дані одночасного впливу аргону, барботованого зі швидкістю 0,2 см<sup>3</sup>/с через водне середовище (об'єм 75 см<sup>3</sup>) та ультразвукової кавітації (частота 22 кГц, потужність 35 Вт) на дріжджі *Saccharomyces cerevisiae* впродовж двогодинної тривалості процесу. Кількість мікроорганізмів в одиниці об'єму досліджуваної води визначалась загальною чисельністю колоній на поживному середовищі на чашках Петрі. Встановлено активне зменшення чисельності клітин на початку процесу (61,84 % після 30 хв) при вихідному мікробіологічному забрудненні води  $2,07 \times 10^4$  КУО/см<sup>3</sup> з досягненням частки загиблих клітин > 98 % після обробки води тривалістю 1 година. Отримані результати свідчать про інтенсивне кавітаційне очищення води від досліджуваних мікроорганізмів при барботуванні аргону.

**Ключові слова:** руйнування дріжджових клітин, ступінь руйнування, дія аргону, кавітація.

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